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Efficacy of Emodepside plus Toltrazuril Oral Suspension for Dogs (Procox®, Bayer) against *Trichuris vulpis* in Naturally Infected Dogs

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Abstract

The efficacy of emodepside plus toltrazuril oral suspension for dogs (Procox®, Bayer) against *Trichuris vulpis* was evaluated in a controlled, blinded and randomised laboratory study. Twenty naturally infected dogs were included. Dogs in the treatment group received the minimum therapeutic dose of 0.45 mg emodepside and 9 mg toltrazuril per kg body weight, while dogs in the control group were left untreated. Efficacy was calculated based on worm counts after necropsy on Day 7 post treatment. Additionally, all faeces were collected and examined for expelled worms. The treatment was 100 % effective. A total of 233 adult worms (geometric mean 17.0) and 3 immature adult worms were found in the control group at necropsy. Adequacy of infection was demonstrated. The treated group excreted a total of 186 adult worms within 2 days after treatment. Additionally, all dogs were co-infected with Uncinaria stenocephala. Efficacy against this parasite was 99.8 %. No side effects of the treatment were observed. This study demonstrates that in addition to the formerly proven efficacy against *Toxocara canis, Ancylostoma caninum* and *Uncinaria stenocephala*, emodepside plus toltrazuril suspension is also effective against *T. vulpis* and thus represents a convenient treatment option for dogs co-infected with whipworms and coccidia.

Introduction

Trichuris vulpis is a blood-feeding intestinal nematode of wild and domestic canids worldwide. Its common name "whipworm" is derived from the adult worm's body shape with a thick, whip handle-like posterior part, pointing into the intestinal lumen, and a long and slender anterior part which is embedded in the mucosa, mainly of the caecum. Continual movement of the head leads to tissue damage and may result in haemorrhagic colitis (Kirkova and Dinev 2005) and severe electrolyte imbalances, in some cases mimicking symptoms of Addison's disease (Ruckstuhl et al. 2002; Venco et al. 2011). The life cycle is direct, and after a prepatent period of 9–10 weeks typical lemon-shaped, highly resistant eggs are released into the environment that may remain infective for several years. After ingestion, the larvae hatch in the small intestine and penetrate the mucosa, provoking desquamation of the epithelium, hyperaemia and mucoid dystrophy. The histotropic phase in the small intestine takes about two weeks before the large intestine is colonised (Kirkova and Dinev 2005). Due to the long prepatent period and egg ingestion being the only source of infection, *T. vulpis* is rarely found in dogs less than three months of age (Barutzki and Schaper 2011). While prevalence can vary regionally and in private dogs mostly does not exceed 5% (Epe et al. 2004; Barutzki and Schaper 2003; Riggio et al. 2013; Sager et al. 2006), values of up to 59.4% can be found in stray dogs and kennel dogs (Capelli et al. 2006).

Emodepside plus toltrazuril suspension (Procox®, Bayer) is a combined nematodicide and coccidiocide for the treatment of dogs, including puppies from two weeks of age, with suspected or demonstrated mixed infections of nematodes (*Toxocara canis, Ancylostoma caninum, Uncinaria stenocephala)* and coccidia (*Isospora ohioensis*-complex, *Isospora canis* [EMA 2011; Altreuther et al. 2011a, 2011b; Schimmel et al. 2011]). Recently, efficacy and safety of the formulation was also demonstrated in cats (Petry et al. 2011a, 2011b). Especially in breeding facilities, co-infection with nematodes and coccidia is a common finding (Seeliger 1999; Bode 1999) and the impact on growth and development may be significant (Daugschies et al. 2000; Junker and Houwers 2000).

The present study was designed to evaluate the efficacy of the emodepside plus toltrazuril suspension against *T. vulpis*.

Materials and methods

This dose confirmation study was conducted as a negative controlled, randomised and blinded laboratory study in accordance with VICH guideline 9 "Good Clinical Practice" (July 2000) and the anthelmintic guidelines VICH GL7 "Efficacy requirements for anthelmintics: general requirements" (December 2000) and VICH GL 19 "Efficacy of anthelmintics: specific recommendations for canines" (July 2001).

Study animals

The study population consisted of 20 naturally infected cross-breed dogs (16 female/4 male, mainly < 12 months old) that were owned by ClinVet International (Pty) Ltd and weighed between 4.8 and 12.8 kg. After an acclimatisation period of seven days, the dogs were randomised to two study groups based on body weight and gender. Each study group contained ten dogs, and uniquely numbered collar tags were used to identify the animals. Fitness for inclusion was demonstrated by at least two faecal egg counts (FEC) of ≥ 60 *T. vulpis* eggs per gram faeces, good health as determined at two clinical examinations performed on Day –7 and –1 and absence of recent treatments with drugs that could influence the study results, especially macrocyclic lactones. As the dogs were naturally infected, co-infections with other parasites were expected. The dogs were individually housed in pens throughout the study. Commercial dog food

(Eukanuba Puppy and Junior – Medium) was fed once per day at the recommended rates and tap water was provided *ad libitum*.

Treatment and health observations

On Day 0, dogs assigned to group 2 were treated with the minimum therapeutic dose of 0.45 mg emodepside and 9 mg toltrazuril per kilogram body weight (b.w.), corresponding to 0.5 ml oral suspension per kg b.w. The appropriate volume was applied using syringes with a 0.1 ml scale and care was taken to ensure that the full dose was swallowed and no substance was lost. Dogs in group 1 served as negative control and were left untreated. Clinical assessments including behaviour, respiration, salivation/vomiting, eyes, locomotion/musculature, feed consumption and faeces were conducted pre treatment, 1, 2 and 4 hours after treatment and once daily on Day 1 and 2. Special attention was paid to vomitus or regurgitation at dosing and additionally 30 minutes after dosing. Observations on the general health of the dogs were performed on all other study days.

Faecal examinations

FEC's for study inclusions were performed using McMaster method. All dogs were carefully monitored for spontaneously excreted worms and worms expelled after treatment. For this purpose, total faeces were collected individually from each dog on a daily basis between Day –3 and Day 7. Using sieving and microscopic evaluation, all worms expelled in the faeces were recovered and preserved. Whenever nematodes other than *T. vulpis* were detected, these were counted and processed in the same manner as the target parasite. Cestodes were only recorded as present or absent, without any quantification.

Necropsy

Euthanasia and necropsy were performed on Day 7. The digestive tract from the beginning of the stomach to the rectum was removed and the small and large intestines were processed separately. The intestinal contents and the results of several mucosal strippings were washed over sieves with apertures of 150 µm and examined under a stereomicroscope. All nematode parasites collected at necropsy were counted and identified to genus, species and developmental stage.

Efficacy determination and statistical analysis

To assess the adequacy of infection in the control group, recommendations in VICH guidelines 7 and 19 were followed. These require a minimum of 6 control animals with at least 5 worms each. Additionally, the lower 95% confidence limit should be greater than 10% of the central tendency. The geometric mean was used for this calculation as all worm counts in the control group were >0.
Percentage efficacy was calculated according to the following formula, recommended by VICH guideline 7 and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994):

$$\%\ \text{Effectiveness (reduction)} = 100 \times \frac{N1 - N2}{N2}$$

N1: geometric mean of worm count for the control group
N2: geometric mean of worm count for the treatment group

Due to the presence of zero-values, all counts were modified by adding 1 prior to log transformation and subtracting 1 from the antilog value. To test for treatment group effects, the data were analysed using the non-parametric Wilcoxon rank sum test (two-tailed, $\alpha = 0.05$).

Results

Mild changes in faecal consistency were occasionally observed in both study groups pre and post treatment and were not considered to be treatment-related. Results of the parasitological evaluations for *T. vulpis* are summarised in Table 1. Requirements for the adequacy of infection in the control group were fulfilled for adult worms. A total

Table 1 Efficacy of emodepside plus toltrazuril suspension against *T. vulpis* in naturally infected dogs

Group	Dog no.	FEC pre treatment (eggs per gram faeces)*	No. of worms expelled post treatment	Worms recovered at necropsy		Efficacy
				No. of adult / immature adult worms	Geometric mean (adult / total)	
Group 1 Control	1	86.7	0	23/0	17.0/17.6	–
	2	93.3	0	13/0		
	3	60.0	0	6/0		
	4	773.3	1	71/0		
	5	340.0	0	30/0		
	6	46.7	0	8/0		
	7	233.3	0	8/0		
	8	166.7	0	40/0		
	9	273.3	0	28/0		
	10	73.3	0	6/3		
Group 2 treatment	11	146.7	15	0/0	0/0	100 %
	12	133.3	56	0/0		
	13	113.3	1	0/0		
	14	126.7	10	0/0		
	15	66.7	5	0/0		
	16	86.7	26	0/0		
	17	93.3	17	0/0		
	18	186.7	10	0/0		
	19	46.7	11	0/0		
	20	260.0	35	0/0		

* Arithmetic mean of three evaluation dates

of 233 adult (range 6 to 71) and 3 immature adult *T. vulpis* were recovered from the control group at necropsy. No worms were recovered in the treated group, thus the efficacy was 100 % ($p < 0.0001$) based on adult and as well as on total worm burdens. From the faeces collected after Day 0, a total of 186 worms were recovered from the treated group and all but one dog excreted five or more worms after treatment (range 1 to 56). Except for 10 worms that were found on Day 2, all worms were recovered on Day 1. No worms were found later than Day 2. In the control group, one spontaneously expelled worm was found on Day 4. During pre treatment faecal examinations, one spontaneously expelled worm was found in the control group and a total of four worms were recovered from three dogs in the treated group.

Besides the target parasite *T. vulpis*, the following helminth species were detected during faecal examinations and at necropsy: *Uncinaria stenocephala, Ancylostoma caninum, Toxocara canis, Dipylidum caninum* and *Taenia* sp. Only *U. stenocephala* was found in meaningful numbers that fulfilled the requirements for the adequacy of infection and allowed for efficacy calculations. A total of 1,993 adult worms (range 80–429) and two

immature adult worms were found in the control group at necropsy (geometric mean = 166.3). Nine of ten treated dogs were negative for *U. stenocephala* at necropsy (geometric mean = 0.3). The calculated efficacy was 99.8 % ($p < 0.0001$). All but one dog expelled 5 or more worms in the faeces after treatment in group 2 resulting in a total of 1,484 *U. stenocephala* (range 1–870).

Discussion

The study presented here demonstrated 100 % efficacy of emodepside plus toltrazuril suspension against adult *T. vulpis* in naturally infected dogs. The oral suspension is the third introduction of emodepside as a novel nematodicide in veterinary medicine. Efficacy of emodepside against *T. vulpis* has already been demonstrated for the tablet formulation "Profender® tablets for dogs" (Bayer) which is a combination product of emodepside and praziquantel. The tablet formulation was 100 % effective against adult *T. vulpis* and 99.9 %/100 % effective against immature adult *T. vulpis* in experimentally and naturally infected dogs (Schimmel et al. 2009).

Emodepside plus toltrazuril suspension is indicated for the treatment of dogs infected with or at risk of mixed infections of nematodes and coccidia. Especially in young puppies, coccidiosis can be fatal, thus this age group is the main target for a combined treatment. Although *T. vulpis* is mainly found in dogs older than three months of age, the demonstrated efficacy of the suspension against this parasite is beneficial. The high reproductive rate of *T. vulpis* together with an extreme resistance of the eggs can result in a heavily contaminated environment, particularly in facilities where dogs have limited roaming space, as sometimes seen in kennels and breeding facilities. Similar epidemiological aspects apply for intestinal coccidia-like *Isospora* sp. Although hygiene measures play an important role, it is unlikely that this parasite can be eliminated from a facility by decontamination. Thus, treatment of carrier animals, represented by older dogs shedding oocytes without displaying clinical symptoms, may additionally be indicated to reduce the infection pressure.

The demonstrated efficacy against *T. vulpis* completes the range of intestinal nematodes affected by emodepside plus toltrazuril suspension. It can be concluded that Procox® oral suspension for dogs provides a unique treatment option against the most common intestinal nematodes whenever a simultaneous treatment against coccidia is indicated.

Ethical standards

The study was performed in compliance with current applicable local laws and regulations.

Conflict of interest

The study was funded by Bayer Animal Health GmbH, Germany. Gabriele Petry and Gertraut Altreuther are employees of Bayer Animal Health. Dawie Kok and Petro Swart are employees of ClinVet International Ltd, which was contracted to manage and monitor the study by Bayer Animal Health. Sonja Wolken contributed to the preparation of this manuscript.

References

Altreuther G, Gasda N, Adler K, Thurieau H, Schimmel A, Hutchens D, Krieger KJ (2011) Field evaluations of the efficacy and safety of emodepside plus toltrazuril (Procox® oral suspension for dogs) against naturally acquired nematode and *Isospora* spp. infections in dogs. Parasitol Res 190 (Suppl 1):S21–S28

Altreuther G, Gasda N, Schroeder I, Joachim A, Settje T, Schimmel A, Hutchens D, Krieger KJ (2011) Efficacy of emodepside plus toltrazuril (Procox® oral suspension for dogs) against prepatent and patent infection with *Isospora canis* and *Isospora ohioensis*-complex in dogs. Parasitol Res 190 (Suppl 1):S9–S20

Barutzki D, Schaper R (2003) Endoparasites in dogs and catsin Germany 1999–2002. Parasitol Res 90(Suppl 3): S148–S150

Barutzki D, Schaper R (2011) Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. Parasitol Res 109 (Suppl 1):S45–S60

Bode K (1999) Endoparasitenbefall in kommerziellen Hundezuchten unter besonderer Berücksichtigung der Isosporose. Dissertation, Tierärztliche Hochschule Hannover

Capelli G, Frangipane di Regalbono A, Iorio R, Pietrobelli M, Paoletti B, Giangaspero A (2006) *Giardia* species and other intestinal parasites in dogs in north-east and central Italy. Vet Rec 159:422–424

Daugschies A, Mundt H-C, Letkova V (2000) Toltrazuril treatment of cystoisosporosis in dogs under experimental and field conditions. Parasitol Res 86:797–799

Epe C, Coati N, Schnieder T (2004) Results of parasitological examinations of faecal samples from horses, ruminants, pigs, dogs, cats, hedgehogs and rabbits between 1998 and 2002. Dtsch Tierärztl Wochenschr 111:243–247

European Medicines Agency (2011) European Public Assessments reports (EPAR): Procox Product information. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/veterinary/002006/WC500106177.pdf

Jacobs DE, Arakawa A, Courtney CH, Gemmell MA, McCall JW, Myers GH, Vanparijs O (1994) World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics in dogs and cats. Vet Parasitol 52:179–202

Junker K, Houwers DJ (2000) Diarrhoea, pup mortality and *Cystoisospora* species (coccidiosis). Tijdschr Diergeneeskd 125:582–584

Kirkova Z, Dinev I (2005) Morphological changes in the intestine of dogs, experimentally infected with *Trichuris vulpis*. Bulg J Vet Med 8:239–243

Petry G, Kruedewagen E, Bach T, Gasda N, Krieger KJ (2011) Efficacy of Procox® oral suspension for dogs (0.1 % emodepside and 2 % toltrazuril) against experimental nematode (*Toxocara cati* and *Ancylostoma tubaeforme*) infections in cats. Parasitol Res 109(Suppl 1):S37–43

Petry G, Kruedewagen E, Kampkoetter A, Krieger KJ (2011) Efficacy of emodepside/toltrazuril suspension (Procox® oral suspension for dogs) against mixed experimental *Isospora felis*/*Isospora rivolta* infection in cats. Parasitol Res 109(Suppl 1):S29–S36

Riggio F, Mannella R, Ariti G, Perrucci S (2013) Intestinal and lung parasites in owned dogs and cats from central Italy. Vet Parasitol 193:78–84

Ruckstuhl N, Hoerauf A, Tomsa K, Reusch C (2002) Pseudohypoadrenocorticism in two Siberian huskies with gastrointestinal parasitoses. Schweiz Arch Tierheilkd 144:75–81

Sager H, Moret CS, Grimm F, Deplazes P, Doherr MG, Gottstein B (2006) Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelminthic treatment. Parasitol Res 98:333–338

Schimmel A, Altreuther G, Schroeder I, Charles S, Cruthers L, Kok DJ, Kraemer F, Krieger KJ (2009) Efficacy of emodepside plus praziquantel tablets (Profender tablets for dogs) against mature and immature adult *Trichuris vulpis* infections in dogs. Parasitol Res 105(Suppl 1):S17–S22

Schimmel A, Schroeder I, Altreuther G, Settje T, Charles S, Wolken S, Kok DJ, Ketzis J, Young D, Hutchens D, Krieger KJ (2011) Efficacy of emodepside plus toltrazuril (Procox® oral suspension for dogs) against *Toxocara canis, Uncinaria stenocephala* and *Ancylostoma caninum* in dogs. Parasitol Res 109(Suppl 1):S1–S8

Seeliger U (1999) Feldstudie zur Epidemiologie und Bekämpfung der Isosporose des Hundes. Dissertation, Tierärztliche Hochschule Hannover

Venco L, Valenti V, Genchi M, Grandi G (2011) A dog with pseudo-addison disease associated with *Trichuris vulpis* infection. J Parasitol Res. doi: 10.1155/2011/682039

VICH guideline 7: Efficacy requirements for anthelmintics: overall guidelines. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, December 2000

VICH guideline 9: Good clinical practice. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, July 2000

VICH guideline 19: Efficacy of anthelmintics: specific recommendations for canine. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, July 2001